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SSRI augmentation strategies

Cremers, Thomas Ivo Franciscus Hubert

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Chapter 7

Is the beneficial antidepressant effect of co-administration of pindolol really due to somatodendritic autoreceptor antagonism ?

Abstract

The combination of SSRIs with the beta adrenoceptor / 5HT_{1A} receptor antagonist pindolol is currently investigated, based on the concept that 5-HT_{1A} receptor blockade would eliminate the need for desensitization of presynaptic 5-HT_{1A} receptors and therefore hasten the onset of action and improve the efficacy of SSRIs. However, since pindolol plasma levels after 2.5 mg t.i.d. are about 60 nM, and the K_i for the 5-HT_{1A} receptor is 30 nM, it is questionable whether pindolol levels in the brain would be sufficient to antagonise 5-HT_{1A} receptors.

Using microdialysis and jugular vein catheterisation, the ability of systemically administered pindolol to antagonise central 5-HT_{1A} and beta adrenoceptors was studied, while simultaneously monitoring pindolol plasma and brain concentration.

Augmentation of paroxetine-induced increases in extracellular 5-HT levels in ventral hippocampus was only observed at steady state plasma levels exceeding 7,000 nM (concurrent brain levels 600 nM). In contrast, antagonism of beta agonist induced increases of brain c-AMP levels was already observed at pindolol plasma levels of 70 nM (concurrent brain levels < 3nM). At plasma levels that are observed in patients after t.i.d. 2.5 mg (~60 nM), pindolol produces only a partial blockade of presynaptic 5-HT_{1A} autoreceptors and does not augment the SSRI-induced 5-HT increase in guinea pig brain. It is therefore very unlikely that the favourable effects of combining pindolol with SSRIs, as reported in a number of clinical studies, are due to 5-HT_{1A} antagonism. Since pindolol completely blocks central beta-adrenoreceptors at clinically relevant plasma levels, it is possible that beta adrenoceptor antagonism is involved in mediating pindolol's beneficial effects.

1. Introduction

Selective serotonin re-uptake inhibitors (SSRIs) are believed to exert their antidepressive effect by increasing the availability of extracellular 5-HT for interaction with post-synaptic 5-HT receptors. However, increased 5-HT levels will also activate cell body 5-HT_{1A} autoreceptors, resulting in reduced cell firing rate and ultimately in decreased terminal 5-HT release. Chronic administration of SSRIs has been shown to desensitise 5-HT_{1A} autoreceptors, thereby relieving the serotonergic neuron of its auto-inhibitory regulation. This sequence of events is thought to be responsible for the slow onset of action of SSRI's, which often requires 3-4 weeks before clinical effects are observed (Blier et al. 1987). Therefore, it was hypothesised that co-administration of an SSRI with a 5-HT_{1A} autoreceptor antagonist would lead to a more rapid onset of antidepressive action (Artigas 1993, Blier et al. 1994). Several clinical trials have indeed shown that combining an SSRI with the mixed beta-adrenoceptor/5-HT_{1A} antagonist, pindolol, might be beneficial in the treatment of depression, although others failed to observe a more rapid onset of action or a superior effect of the combination (McAskill et al. 1998, Nelson 2000). The use of various SSRIs and diverse inclusion criteria for the trials might at least partially explain these discrepancies. However, it is obvious that pharmacokinetic considerations should be taken into account as well, since the augmentation of the SSRI evoked increase in extracellular 5-HT levels in rat or guinea pig brain is known to be dependent on the dose, and consequently brain levels, of both the SSRI and the 5-HT_{1A} receptor antagonist (Hjorth et al. 1997, Cremers et al. 2000).

In the majority of the animal studies the minimal pindolol dose required to augment the SSRI effect on 5-HT levels ranges from 8 to 15 mg/kg i.p. or s.c. (Dreshfield et al. 1996, Hjorth 1996, Romero et al. 1996). Although the elimination half-life of pindolol in rodents is much faster than in humans (20 min vs. 3.5 h), these doses will still induce very high plasma levels. Based on a volume of distribution of 7.6 L/kg for the active enantiomer in rats and assuming that subcutaneous absorption is not rate limiting, 8-15 mg/kg (-) pindolol will give initial plasma concentrations of approximately 6 μ M ($C_i = \text{Dose}/V_d$; Hasegawa et al. 1989). These plasma levels are two orders of magnitude higher than those found in patients undergoing combination therapy with SSRIs and 2.5–5 mg t.i.d. pindolol, when plasma levels are found to be 30-60 nM (Moffat et al. 1986, Hasegawa et al. 1989, Perez et al. 1999). Because pindolol is a rather polar compound ($\log P = -0.9$; Moffat et al. 1986) and 50% protein bound (Belpaire et al. 1982), it will not easily penetrate into the brain and free pindolol concentrations in the brain are expected to be much lower than plasma concentrations. With a K_i for the 5-HT_{1A} receptor in the same range as the clinical plasma concentrations (30 nM (Boddeke 1992)), it is therefore very unlikely that the brain levels of pindolol after 2.5-5 mg t.i.d. are high enough to block central presynaptic 5-HT_{1A} receptors in the raphe nuclei.

The present study addresses these inconsistencies by examining the pharmacokinetics and pharmacodynamics of pindolol in guinea pigs, during co-administration with the SSRI paroxetine. We determined to what extent different pindolol plasma levels affected the paroxetine-induced increase in extracellular 5-HT in rat ventral hippocampus, as an estimate of the degree of 5-HT_{1A} receptor blockade. In addition, since pindolol is a more potent beta-adrenoceptor antagonist ($K_i = 2$ nM) than 5-HT_{1A} antagonist, we also determined the effect of

pindolol administration on the beta-receptor agonist-induced stimulation of c-AMP formation (Beer et al. 1988). During these experiments plasma levels of pindolol and paroxetine, as well as pindolol brain levels in the raphe nuclei, were measured to correlate actual brain and plasma levels with the pharmacological effects. Since pindolol has considerable affinity for rat 5-HT_{1B}, but not for human and guinea pig 5-HT_{1B} receptors, the present study was performed in freely moving guinea pigs in order to prevent interference by 5-HT_{1B} antagonism of pindolol during SSRI administration (Moret et al. 1997, Zgombick et al. 1997). In addition, the affinity of (-)-pindolol for guinea pig and human 5-HT_{1A} receptors is near similar (Raurich et al. 1999)

2. Materials and Methods

2.1 Animals and drugs

Male albino guinea pigs of a Dünkink Hartley strain (300-400 g; Harlan, Zeist, The Netherlands) were housed in cages (32 x 40 x 40 cm) with free access to food and water. All experiments were concordant with the declarations of Helsinki and were approved by the Animal Care Committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

The following drugs were used: paroxetine (SKB, West Sussex, UK), (\pm) pindolol, (\pm)-isoprenaline (RBI, Natick, USA) and dibucaine (Sigma, St. Louis, USA). Paroxetine was dissolved in water and injected subcutaneously. Pindolol was dissolved in glacial acetic acid (10,100 and 1000 mg/ml), diluted with saline to 0.1, 1 and 10 mg/ml and adjusted to pH 5.5.

Isoprenaline solutions for local administration were freshly prepared daily in a concentration of 10 μ M in Ringer's solution containing 5 μ M of ascorbic acid to prevent oxidation of isoprenaline.

2.2 Surgery and microdialysis

Guinea pigs were anaesthetised with ketamine/xylazine (50/8 mg/kg), after premedication with midazolam (5 mg/kg s.c.) and using lidocaine-HCl 10 % (m/v) for local anaesthesia. The animals were placed in a stereotaxic frame (Kopf, USA) and home-made I-shaped probes were implanted and secured with dental cement, using the following co-ordinates Luparello (1965) for 5-HT and cAMP measurements in ventral hippocampus: anterior from intra-aural + 4.9 mm, lateral from midline \pm 6.5 mm, ventral from dura - 9.0 mm; for pindolol measurements in the raphe nuclei: anterior + 2.8 mm, lateral + 2.6 mm ventral - 11.0 mm at an angle of 12°. Probes for serotonin and cAMP measurements were constructed with a polyacrylonitrile/sodium methyl sulphonate copolymer dialysis fibre (4 mm open surface, i.d. 220 μ m, o.d. 310 μ m, AN 69, Hospal, Italy), for pindolol measurements with a cellulose membrane (5 mm open surface, i.d. 200 μ m, Spectra/Por®, Hollow fiber bundles) (Santiago & Westerink 1990). A polyethylene canula (8 cm, i.d. 0.5 mm, o.d. 1.0 mm) was positioned subcutaneously in the neck region for pindolol infusion and a silicon canula was inserted 4.2 mm into the right jugular vein for blood sampling. The tubing was guided subcutaneously to the skull and connected with a stainless steel inlet, which was mounted on the skull with dental cement and surgical screws. After insertion, the canula was filled with a PVP solution (55 % polyvinylpyrrolidone in 500 IE/ml heparin) in saline to prevent blood clotting. Postoperative analgesia was accomplished by an intramuscular injection of 0.1 mg/kg buprenorphine and the animals were allowed to recover for at least 24 hours.

At the day of the experiment, microdialysis probes were connected with flexible PEEK tubing to a microperfusion pump (Harvard apparatus, South Natick, MA, USA) and perfused with artificial cerebrospinal fluid (aCSF), containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl₂, and 1.2 mM MgCl₂. Flow rates were 1.5 μ l/min for the measurement of extracellular 5-HT and c-AMP and 0.1 μ l/min for pindolol.

2.3 Effect of pindolol on paroxetine-induced 5-HT increase

The effects of two doses of paroxetine (0.5 and 5 mg/kg s.c.) on extracellular 5-HT levels were determined during continuous s.c. infusions with saline (controls) or with increasing concentrations of pindolol. The infusions started 3 hours prior to drug administration. The pindolol infusions were chosen to give steady state plasma levels of approximately 70, 700 and 7,000 nM. Fifteen-minute dialysate samples were collected in vials containing 7.5 µl of 0.02 mM acetic acid.

2.4 Effect of pindolol on isoprenaline-induced c-AMP increase

A 10 µM isoprenaline solution in aCSF was perfused in the ventral hippocampus via the microdialysis probe, during continuous s.c. infusion with saline or with increasing concentrations of pindolol as described above. Microdialysate samples (45 µl) were collected in glass vials.

2.5 Pindolol brain concentrations

In a separate experiment, pindolol concentrations were measured in microdialysates from the raphe nuclei for each of the infusion conditions described above. Although extracellular brain concentrations of exogenous substances are conveniently determined by microdialysis, a correction for the in vivo recovery of the probe is required (Menacherry 1992). By using an ultra-slow flow rate of 0.1 µl/min, which gives approximately 100% in vivo recovery, no exogenous markers are needed to correct for in vivo recovery. We found that the cellulose dialysis membrane rapidly followed pindolol concentration changes in the external medium and that the in vitro recovery approached 100 % at this flow rate. Microdialysate concentrations are therefore not corrected for recovery. Probes were perfused at 0.1 µl/min with aCSF, while pindolol was continuously infused via the s.c. canula. 60 Min dialysate samples were collected on-line in a 20 µl HPLC loop and assayed by HPLC and electrochemical detection.

2.6 Plasma concentrations of pindolol and paroxetine

Blood samples (0.3 ml) were drawn via the jugular vein canula at different time points to determine steady state conditions for pindolol or the time-concentration profile of paroxetine. Samples were collected in 1.5 ml eppendorf vials, containing 5 µl heparin (500 IE/mL saline), mixed and centrifuged for 15 min at 3,000 rpm and 4°C (MSE, England). Aliquots of the supernatants were assayed for pindolol and paroxetine.

2.7 Assays

5-HT: Twenty-µl microdialysate samples were injected via an autoinjector (CMA/200 refrigerated microsampler, CMA, Sweden) on a 100 x 2.0 mm C18 Hypersil 3 µm column (Bester, Amstelveen, the Netherlands) and separated with a mobile phase consisting of 5 g/L di-ammoniumsulfate, 500 mg/L EDTA, 50 mg/L heptane sulphonic acid, 4 % methanol v/v,

and 30 µl/L of triethylamine, pH 4.65 at 0.4 ml/min (Shimadzu LC-10 AD) 5-HT was detected amperometrically at a glassy carbon electrode at 500 mV vs Ag/AgCl (Antec Leyden, Leiden, The Netherlands). The detection limit was 0.5 fmol 5-HT per 20 µl sample (signal to noise ratio 3).

Paroxetine: Plasma paroxetine levels were measured after liquid-liquid extraction with diethylether. To 250 µl plasma samples 75 µl of a 5 µg/ml dibucaine solution in 1 % m/v NaHCO₃ was added. Samples were extracted thrice by mechanically shaking for 3 minutes with 4 ml of diethyl ether. The ether layers were then transferred to 10 ml evaporating tubes, and 150 µl of 0.1 N HCl was added. The ether was evaporated in a water-bath at 40 °C under a stream of nitrogen. The HCl layer was washed once with 0.5 ml ether and 50 µl samples were injected onto the column. An HPLC/auto-injector (1084B Liquid Chromatograph, Hewlett-Packard) was used, in combination with a fluorescence detector (470 Scanning Fluorescence detector, Waters, England) operating at an absorption wavelength of 295 nm, an emission wavelength of 365 nm, and a slit-width of 12 nm. Paroxetine was separated over a Supelcosil HPLC column (5 µm, C18, 250 x 46 mm, Supelco, the Netherlands) using a mobile phase consisting of 46 % v/v acetonitrile, 54 % v/v potassium dihydrogen phosphate buffer (4.3 g/l), and 30 µl/l triethylamine, pH 3.0, at 1 ml/min. Recovery of paroxetine and the internal standard was approximately 100 %, and concentrations were calculated using the internal standard. The detection limit of the assay was 8 nM (signal to noise = 3).

Pindolol in plasma: Pindolol plasma concentrations were also determined after liquid-liquid extraction with diethylether. To 100 µl of plasma, 50 µl of 1 N NaOH was added and samples were extracted thrice by mechanically shaking for 3 minutes with 4 ml of diethyl ether. The combined organic layers were evaporated into 100 µl of 1 mM HCl and 20 µl samples were injected onto the column. The detection limit of the assay was 1 nM (signal to noise = 3). Recovery of pindolol, determined by extracting plasma samples spiked with known concentrations, was about 95 % and all samples were corrected for recovery. Analysis was performed as described below.

Pindolol in microdialysates: Pindolol concentrations in raphe nuclei dialysate were measured by HPLC-ECD. Samples were collected on-line and injected automatically every 60 min and separated over a Supelcosil column, with a mobile phase consisting of 4.27 g/l sodium acetate, 221 mg/l tetramethyl ammonium, 100 mg/l ethylenediaminetetra-acetic acid, and 10 % v/v acetonitrile, pH value 4.25, at 1 ml/min (LKB 2150, Pharmacia LKB, Sweden). Pindolol was detected amperometrically at a glassy carbon electrode set at 850 mV vs Ag/AgCl (Antec Leyden, Leiden, the Netherlands). The detection limit for pindolol was 3 nM.

c-AMP: Concentrations of cAMP in microdialysates from the ventral hippocampal were determined as described by Svensson et al. (Svensson et al. 1990). Briefly, 10 µl of 0.5 M sodium acetate and 10 µl 55% chloroacetaldehyde (Fluka) were mixed with 45 µl microdialysate in vials which were capped, heated in a boiling water bath for 20 min, injected (1084B Liquid Chromatograph, Hewlett-Packard) onto a Supelcosil guard plus analytical column (3C18 3 µm, 15 cm x 2.1 mm; Security Guard, Phenomenex, Bester, Amstelveen, the Netherlands). c-AMP was eluted with 1mM tetramethylammonium, 9 % tetrahydrofuran, pH 4.5, at a flow rate of 0.4 ml/min and detected fluorimetrically (at absorption and emission

wavelengths of 275 nm and 418 nm, respectively (slit-width 12 nm)). The limit of detection was 10.5 fmol/sample (signal to noise =2).

2.8 Protein binding

Protein binding of pindolol was determined by spiking guinea pig plasma with known concentrations of pindolol dissolved in phosphate buffer pH 7.6. After a 1 hour incubation at room temperature, samples were transferred to ultrafiltration tubes (Amicon, cut-off 3 kD), and spun at 1000 rpm for 30 minutes. The free fraction was determined by HPLC-ECD as described above.

2.9 Data presentation and statistics

Four consecutive microdialysis samples with less than 20 % variation in levels of 5-HT and c-AMP were taken as baseline levels and set at 100 %. Drug effects were expressed as percentages of basal level (mean \pm SEM). Statistical analysis was performed using Sigmastat for windows (Jandel Corporation). Treatment effects were compared versus saline treatment using two way ANOVA for repeated measurements, followed by the Student Newman Keuls post-hoc test . Treatment effects were compared versus control values using one way ANOVA for repeated measurements on ranks. The level of significance level was set at $p < 0.05$.

3. Results

3.1 Pharmacokinetics of pindolol and paroxetine

Subcutaneous infusion of pindolol resulted in steady state plasma levels at approximately 3 hours after initiation of the infusions. Infusions with 0.1 mg/kg/ml/h produced stable pindolol plasma levels of about 70 nM. Ten-fold increases in the infusion concentration of pindolol (1 mg/kg/ml/h and 10 mg/kg/ml/h) resulted in ten-fold higher plasma levels to approximately 700 and 7,000 nM, respectively (figure 1).

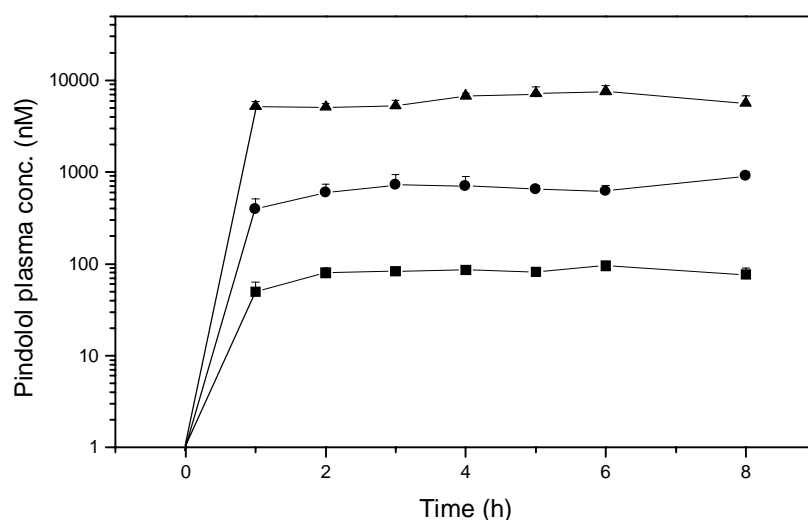


Figure 1. Plasma levels of pindolol during subcutaneous infusion. ■ pindolol 0.1 mg/kg/ml/h (n=3), ● pindolol 1 mg/kg/ml/h (n=4), and ▲ pindolol 10 mg/kg/ml/h (n=5).

The plasma protein binding of pindolol was about 50% over a range of concentrations (n = 2-4, fig 2a) and did not change in the presence of 1 μ M paroxetine (n = 2, fig 2b).

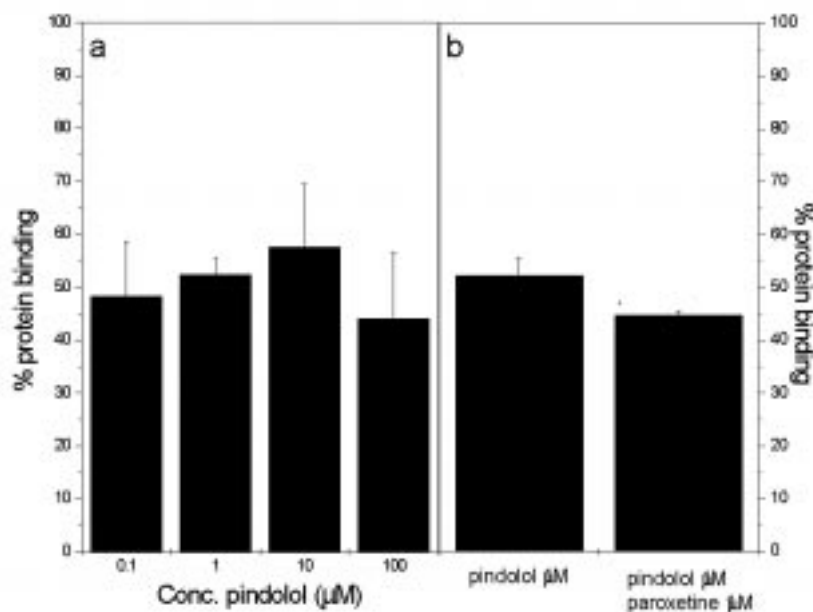


Figure 2. (a) percentage protein binding of pindolol at several concentrations (n=2-4), (b) percentage protein binding of pindolol at 1 µM concentration, with and without the presence of paroxetine 1 µM (n=4).

During pindolol steady state plasma concentrations of 70 nM, pindolol was not detectable in dialysates from the raphe nuclei (< 3 nM). However, at higher pindolol plasma concentrations, the extracellular brain levels of pindolol were approximately 30 nM during 1 mg/kg/ml/h infusion, and 600 nM during 10 mg/kg/ml/h infusion (figure 3). Administration of 5 mg/kg s.c. paroxetine during 10 mg/kg/ml/h pindolol infusions, had no effect on the plasma or brain levels of pindolol (figure 4a-b).

Subcutaneous administration of paroxetine rapidly induced measurable plasma concentrations of the drug. Peak plasma levels after 0.5 mg/kg s.c. paroxetine were 0.5 µM, which declined with an elimination half live of about 75 min. After 5 mg/kg paroxetine s.c. peak plasma levels were 2 µM, after which elimination was bi-phasic, with apparent elimination half lives of 220 min and 800 min, respectively (fig 5).

3.2 Effect of pindolol on the paroxetine-induced 5-HT increase

Absolute output of 5-HT from guinea pig ventral hippocampus was 8.79 ± 0.67 fmol per 15 minute sample (n=38).

Subcutaneous administration of 0.5 mg/kg paroxetine during saline infusion increased hippocampal 5-HT levels about 2-fold. When 0.5 mg/kg paroxetine was administered during pindolol infusions to give steady state plasma concentrations of 70 or 700 nM pindolol, no augmentation of the SSRI effect was observed ($F_{1,140}=0.68$ and $F_{1,129}=1.65$, respectively). However, during steady state levels of 7,000 nM pindolol (10 mg/kg/ml/h s.c.), the effect of paroxetine on extracellular 5-HT levels was significantly potentiated to a 3-fold increase ($F_{1,128}= 2.86$; figure 6).

Essentially the same results were obtained with a 10 times higher dose of paroxetine. The 5-HT increase induced by 5 mg/kg s.c. paroxetine was about 3.5-fold and was unchanged at steady state pindolol levels of 70 nM ($F_{1,101}=0.57$) and 700 nM ($F_{1,116}=0.58$), but was significantly greater when pindolol plasma levels were 7,000 nM ($F_{1,117}=4.37$; figure 7).

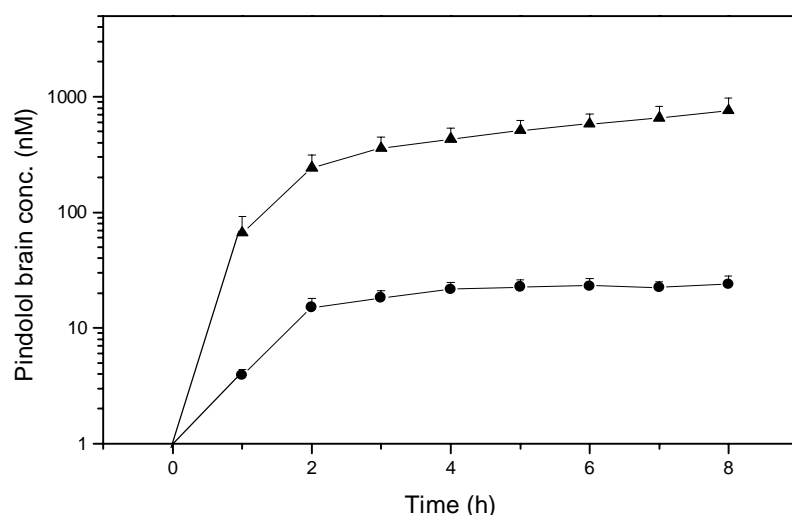


Figure 3. Brain levels of pindolol during subcutaneous infusion. ● pindolol 1 mg/kg/ml/h ($n=3$), and ▲ pindolol 10 mg/kg/ml/h ($n=3$). Brain levels of 0.1 mg/kg/ml/h infusion of pindolol could not be detected.

3.3 Effect of pindolol on the isoprenaline-induced c-AMP increase

Basal extracellular cAMP levels in microdialysates from ventral hippocampus were 739.0 ± 157.6 fmol/sample ($n=8$). These basal levels were somewhat higher than reported in previous studies (Stone 1988), probably because of experimental differences, such as dialysis surface, membranes, brain area and species. Continuous local administration of 10 μ M of the beta adrenoceptor agonist isoprenaline via the hippocampal probe produced significant increases in extracellular cAMP levels. A maximal 2-fold increase over basal levels was reached 3 hours after drug administration ($X^2_6=13.3$, $p<0.05$). When isoprenaline (10 μ M) was administered in the presence of pindolol, at steady state levels of 70 nM, the increase in c-AMP formation was completely blocked ($F_{1,61}=11.13$; figure 8).

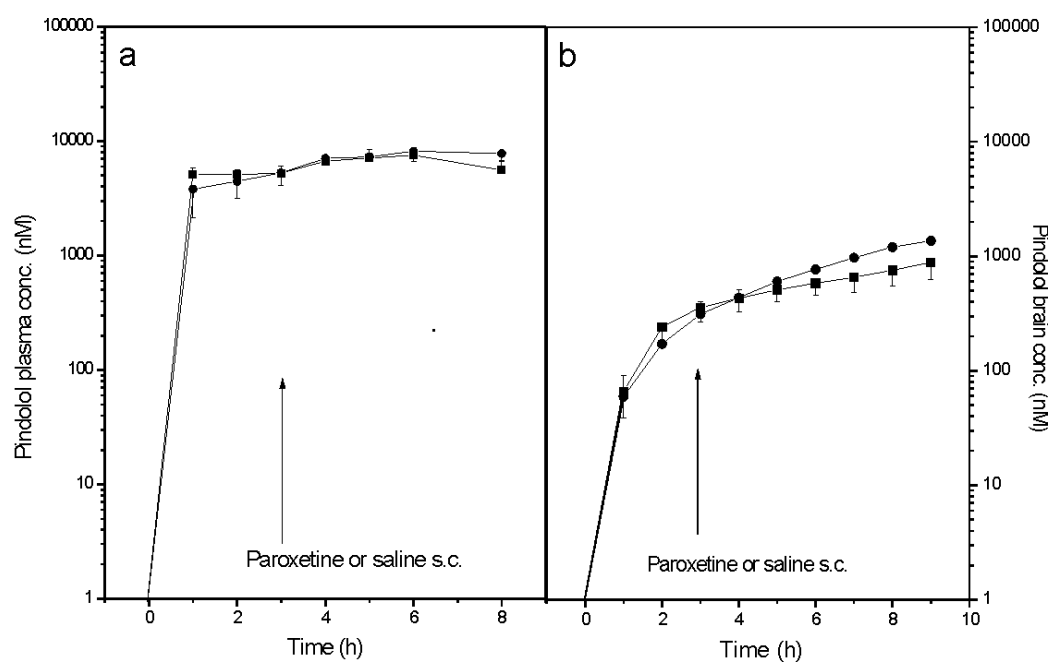


Figure 4. (a) Effect of paroxetine 5 mg/kg s.c. on plasma levels of pindolol during subcutaneous infusion of 10 mg/kg/ml/h pindolol (■ saline injection (n=3), ● paroxetine 5 mg/kg s.c. (n=3)) (b) Effect of paroxetine 5 mg/kg s.c. on brain levels of pindolol during subcutaneous infusion of 10 mg/kg/ml/h pindolol (■ saline injection (n=3), ● paroxetine 5 mg/kg s.c. (n=3))

4. Discussion

Several clinical studies have investigated the putative beneficial effects of co-administration of a 5-HT_{1A} antagonist with antidepressive SSRI treatment, based on the hypothesis that 5-HT_{1A} receptor blockade will hasten the onset of the effects of SSRIs (see Introduction). Although some studies with the beta-adrenergic/5-HT_{1A} receptor antagonist pindolol found evidence for enhanced efficacy of pindolol-SSRI combinations (McAskill et al. 1998, Nelson 2000), pindolol plasma levels after 2.5-5 mg t.i.d. (30-60 nM), seem insufficient to effectively block central 5-HT_{1A} autoreceptors, and consequently to augment the SSRI-induced 5-HT increase. To investigate at what plasma or brain levels pindolol affects the serotonergic effects of SSRIs in more detail, the present study determined the effects of increasing plasma pindolol concentrations on the paroxetine-induced 5-HT increase in guinea pig hippocampus.

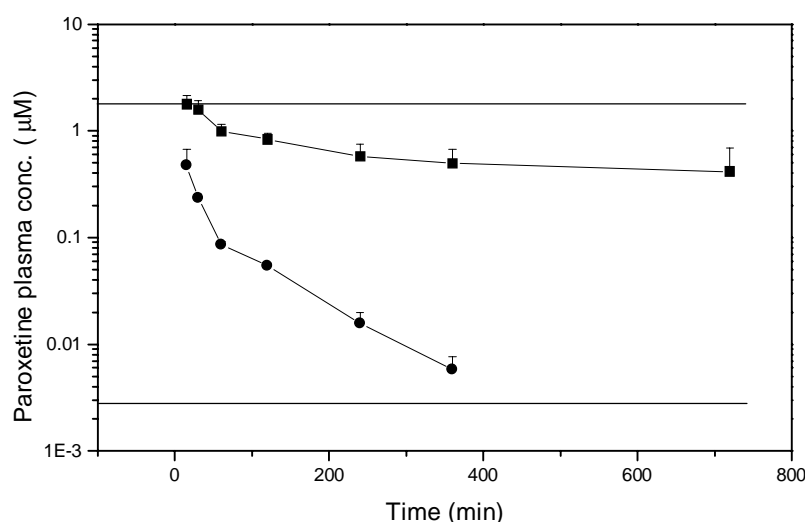


Figure 5. Plasma levels of paroxetine upon subcutaneous administration of 0.5 mg/kg (●, n=3) and 5 mg/kg (■, n=4)

Pindolol augmentation of the effect of paroxetine

Continuous subcutaneous infusions with 0.1, 1.0 and 10 mg/kg/ml/h pindolol, resulted in stable pindolol plasma levels of 70, 700 and 7,000 nM, respectively. Pindolol metabolites, which have negligible affinity for 5-HT receptors and are thus not expected to have any pharmacological effect in patients (Ohnhaus et al. 1982), were not detected under these conditions. During each of the three pindolol steady state conditions, guinea pigs were challenged with two subcutaneous paroxetine doses, 0.5 and 5.0 mg/kg, and the increase in extracellular 5-HT levels was compared with the increase produced by paroxetine during s.c.

infusion with saline. The two doses of paroxetine were chosen to cover the entire range of plasma concentrations of paroxetine observed in depressed patients in the clinic (Dechant et al. 1991; Baumann 1992). Paroxetine metabolites, which have low affinity for the 5-HT transporter, were not detected after administration of either dose (Baumann 1992).

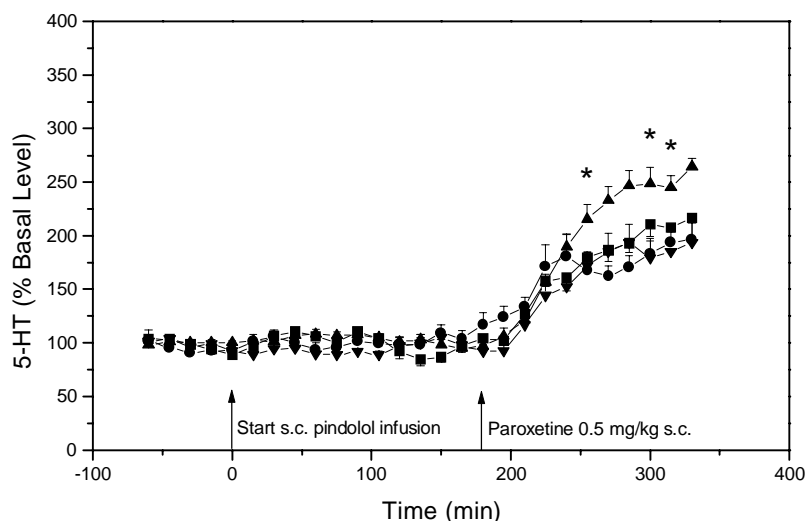


Figure 6. Effect of pindolol pretreatment on paroxetine 0.5 mg/kg s.c. induced increase in extracellular levels of 5-HT. ▼ saline 1 ml/kg/h (n=7), ■ pindolol 0.1 mg/kg/ml/h (n=6), ● pindolol 1 mg/kg/ml/h (n=6), and ▲ pindolol 10 mg/kg/ml/h (n=5), * $P < 0.05$.

At steady state plasma levels of 70 and 700 nM, pindolol failed to change the effect of either dose of paroxetine on extracellular 5-HT levels. Only when pindolol plasma levels were increased to 7,000 nM, the effects of both doses of paroxetine were significantly greater. These findings are in good agreement with several reports on the augmentation of the SSRI-induced 5-HT increase by pindolol co-administration in rats, which is only observed at pindolol doses exceeding 8 mg/kg s.c. (Dreshfield et al. 1996; Hjorth 1996; Romero et al. 1996). Doses of 8 mg/kg s.c. will result in pindolol plasma concentrations of approximately 6,000 nM (see Introduction), which is in the same range as we found to be required for potentiation of the SSRI effect by pindolol. It might be questioned whether similar pharmacokinetic-pharmacodynamic relations would be observed when serotonin levels would be measured in terminal areas other than ventral hippocampus. As no data exist on regional differences in dose-dependency of 5-HT_{1A} antagonist induced augmentation, additional research should elucidate this topic. However, since antagonism of 5-HT_{1A} receptors would in theory be a concentration dependent process, and not like the effects of SSRI's a composition of re-uptake inhibition, and autoreceptor functionality, no differences would be expected. Microdialysis measurements of the actual free brain concentrations of pindolol at its proposed site of action, the raphe nuclei where presynaptic 5-HT_{1A} autoreceptors are located, confirmed that pindolol, which is hydrophilic ($\log P = -0.90$) and 50% protein-bound, does not readily cross the blood-brain-barrier. Brain levels at steady state plasma levels of 70

nM were not detectable (<3 nM), whereas at plasma concentrations of 700 and 7,000 nM, pindolol extracellular levels in the raphe were about 30 nM and 600 nM, respectively.

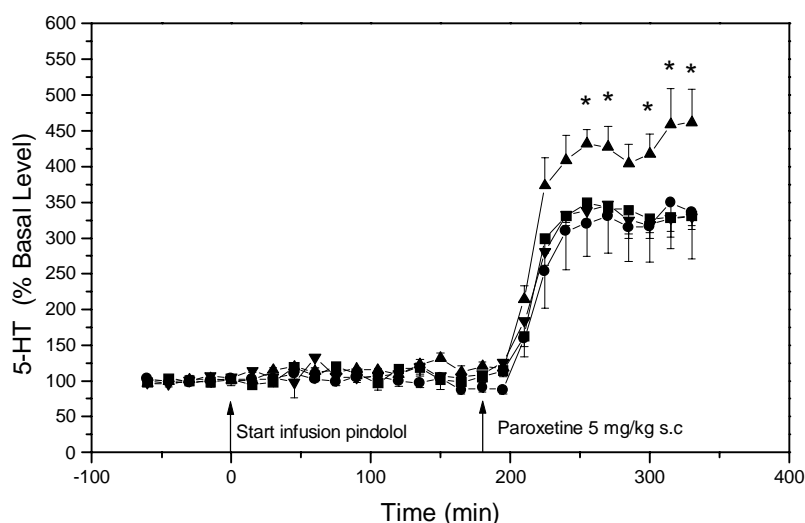


Figure 7. Effect of pindolol pretreatment on paroxetine 5 mg/kg s.c. induced increase in extracellular levels of 5-HT. ▼ saline 1 ml/kg/h (n=6), ■ pindolol 0.1 mg/kg/ml/h (n=4), ● pindolol 1 mg/kg/ml/h (n=5), and ▲ pindolol 10 mg/kg/ml/h (n=5), * P<0.05.

Since it has been suggested that pharmacokinetic interactions between SSRIs and pindolol could possibly lead to changes in drug levels, we also determined the effect of paroxetine on pindolol plasma and brain levels. Since we found no evidence for interaction between the two drugs at the plasma levels studied, and no effect on the protein binding of pindolol, it is unlikely that the beneficial effects of pindolol addition is correlated with increased pindolol levels.

Implications for 5-HT_{1A} receptor occupancy

The finding that pindolol only enhances the paroxetine-induced 5-HT increase at plasma levels that are 100-fold higher than clinical pindolol plasma levels, suggests that it is highly unlikely that SSRI augmentation is due to blockade of presynaptic somatodendritic 5-HT_{1A} autoreceptors. At clinical plasma levels of 30-60 nM (Moffat et al.1986; Hasegawa et al.1989, Perez 1999), pindolol concentrations in the raphe are ≤ 1 nM and will occupy less than 10% of the 5-HT_{1A} receptors, insufficient to block their activation by the endogenous agonist, 5-HT (see below). This estimate is in reasonable agreement with a recent PET study in volunteers who were not receiving SSRI treatment (Andree et al.1999). After a single dose of 10 mg (-) pindolol and at plasma levels of 113-153 nM, the 5-HT_{1A} receptor occupancy in the dorsal raphe ranged from 7-25%. It is however likely that receptor occupancy during combination therapy with 2.5 mg t.i.d. pindolol will be markedly less, since plasma levels will be 2-4 fold lower, while at the same time endogenous 5-HT levels will be elevated in the presence of the SSRI. This is easily demonstrated by extrapolating IC₅₀ values for pindolol in

the absence and presence of an SSRI with the equation, $IC_{50}=K_i(1+C_{ag}/K_{Dag})$ (Cheng & Prusoff 1973). Assuming that brain concentrations of the agonist, 5-HT, are about 1.5 nM (Adell et al. 1991), the IC_{50} of pindolol for the 5-HT_{1A} receptor is calculated to be 42 nM during basal conditions (K_D 5-HT=3.8 nM (Peroutka 1986), K_i pindolol =30 nM (Boddeke 1992)). After SSRI administration, endogenous 5-HT concentrations are 2-3.5 times higher and under these conditions the equation predicts that the IC_{50} is about 54-71 nM and that the receptor occupancy will be lower than found under basal conditions.

Our finding that free pindolol raphe concentrations should be about 600 nM, or 20 times higher than the K_i , to augment the SSRI effect, is consistent with the fact that a strong receptor occupancy is required for functional 5-HT_{1A} receptor antagonism. This is supported by the finding that maximal inhibitory concentrations of pindolol at the human 5-HT_{1A} receptor are also approximately 600 nM (Raurich et al. 1999). In addition, although pindolol was originally assumed to be a full 5-HT_{1A} receptor antagonist, accumulating evidence suggests that pindolol has actually the properties of a partial agonist (De Vivo & Maayani S. 1990, Newman-Tancredi et al. 1998, Fornal et al. 1999, Sprouse et al. 1998) which may further explain the absence of augmentation at low pindolol concentrations. Taken together, these observations are in line with the proposed existence of a substantial 5-HT_{1A} receptor reserve in the raphe nuclei (Cox et al. 1993), which demands strong receptor occupation by an antagonist or partial agonist to reduce the effects of an agonist, in particular a full agonist such as 5-HT, on 5-HT_{1A} autoreceptors.

Other receptor mechanisms: beta-adrenoceptor blockade

Since at clinical plasma levels the pindolol brain concentrations are insufficient to completely block 5-HT_{1A} autoreceptors, other receptor mechanisms that are sensitive to low plasma and brain levels of pindolol may be involved in the augmentation of the antidepressive effects of SSRIs. The very high affinity of pindolol for the beta-adrenoceptor ($K_i=2$ nM), prompted us to investigate one possible other mechanism, central beta-adrenoceptor blockade. We assessed the functional beta-antagonist potency of pindolol in vivo via beta-adrenoreceptor mediated effects on c-AMP formation. Beta-adrenoceptor stimulation has been shown to produce increases in extracellular brain levels of cAMP, an effect that could be blocked by selective beta antagonists, demonstrating the beta adrenergic origin of the c-AMP sampled by microdialysis (Petersen et al. 1996; Egawa et al. 1988). We also found that local infusions with 10 μ M of the beta-adrenoceptor agonist isoprenaline in the ventral hippocampus produced 2-fold increases in extracellular c-AMP levels. When the same concentration isoprenaline was administered during continuous s.c. pindolol infusion (0.1 mg/kg/ml/h) that gave steady state plasma concentrations of 70 nM, the c-AMP increase was completely abolished. Whereas plasma levels of 70 nM pindolol thus failed to augment the serotonergic effect of an SSRI, the same pindolol concentrations were able to completely inhibit the increase in c-AMP levels produced by local infusion with a beta-adrenoceptor agonist. This demonstrates that at clinically relevant plasma levels of pindolol, beta adrenoceptors are functionally blocked. Antagonism of beta-adrenoceptors could be clinically relevant as

several authors have shown that beta-adrenoceptors desensitise during treatment with antidepressants (Petersen et al. 1996). Co-administration of SSRIs with beta antagonists would then mimic this desensitisation and therefore might be beneficial for the treatment of depression.

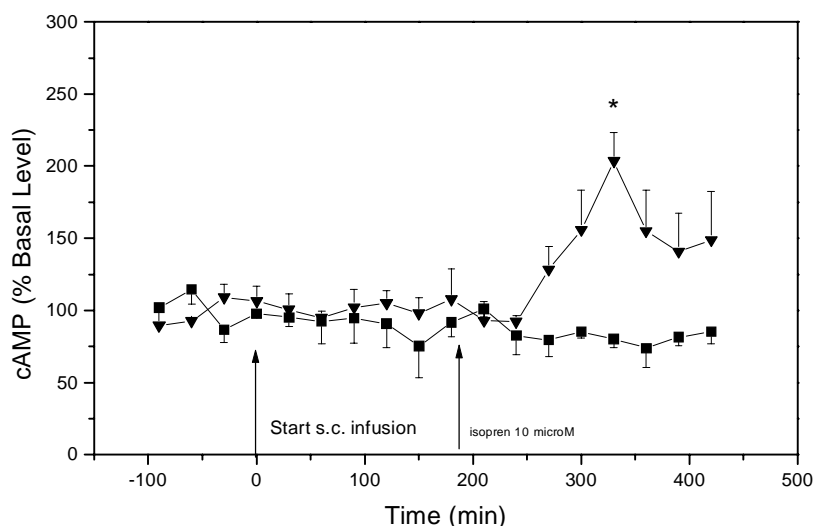


Figure 8. Effect of pindolol 0.1 mg/kg/ml/h pretreatment on local infusion of isoprenaline (10 μ M) induced increase in extracellular levels of cAMP. ▼ saline 1 ml/kg/h (n=4), ■ pindolol 0.1 mg/kg/ml/h (n=4), * P<0.05.

In conclusion, the present study provides pharmacokinetic and pharmacodynamic evidence that pindolol, at the plasma levels observed in clinical studies with 2.5-5 mg t.i.d. pindolol, does not augment the 5-HT increasing effect of the SSRI paroxetine in the guinea pig and that at these levels 5-HT_{1A} autoreceptors in the raphe nuclei are only partially occupied. In addition, pindolol plasma and brain levels are not affected by the simultaneous administration of paroxetine. Therefore, the putative beneficial effects of co-administration of pindolol with an SSRI are likely mediated via other receptor mechanisms, such as for instance central beta-adrenoreceptor blockade, since beta-adrenergic receptors are completely blocked by pindolol at clinically relevant plasma levels.

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